THE EMBRYONIC DEVELOPMENT OF THE OVARY AND TESTIS OF THE MAMMALIA. (PRELIMINARY ACCOUNT.)

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The following is a preliminary account of work begun in the spring of 1900, upon the subject of the development of the ovary and testis of the mammalia. The rabbit and pig have served as the subjects for this work. The results will here be very briefly set forth, a more detailed account being reserved for a later paper. The material studied includes various stages in the development of the ovary and testis of the rabbit, from the thirteen-day embryo to and including adult stages. The pig material includes only embryonic stages, but is more complete for the period covered than is the rabbit material.

The work was carried on with the aim of solving the following problems: (1) the origin and development of the seminiferous tubules and their homologues in the ovary; (2) the origin, development, and homologies of the rete tubules, also their relations to the Malpighian corpuscles of the mesonephros on the one hand, and to the seminiferous tubules of the testis and medullary cords of the ovary on the other; (3) the origin, development and homologies of the connective tissue elements and interstitial cells of the ovary and testis.

Incidental to the solution of these problems, the work has involved to a greater or less extent, a consideration of the following allied problems: (1) the development of sex cells; (2) the morphological phases of sex differentiation; (3) cell degeneration in the sex gland and rete region, (4) the degeneration of the mesonephros, and the development of the Wolffian and Müllerian ducts.

The results of work in so large a field can be, only to a limited extent, new. Certain of the following results are confirmatory of the work of other authors, to whose results I shall refer in a later paper. The very contradictory opinions met with in the literature on the subject call for confirmatory evidence upon these problems.

The earliest rudiment of the sex gland is situated in the genital ridge, which consists of a zone of thickened peritoneum, running the entire length of the mesonephros, parallel and close to the mesentery which unites the latter to the body wall. The rete is formed from the anterior part of the genital ridge and extends, in the pig, from about the region of the sixth glomerulus to a point about opposite the twentieth glomerulus, as shown in a number of series. However, these limits are variable. In the rabbit the limits are more constant, but still variable, the rete extending approximately from the sixth to the twelfth glomerulus. The anterior end of the sex gland rudiment slightly overlaps the posterior end of the rete region. Posterior to the sex gland is a section of the genital ridge that does not develop beyond the very early formation of a region of thickened peritoneum. In each of these three zones of the genital ridge are found scattered cells with distinct cell walls, clear cytoplasm, large round nucleus, centrosphere and centrosome — the so-called primitive sex cells.

In its early stages of development the genital ridge consists of a thickened layer of peritoneum overlying a loose mesenchyma; the cells of the latter are to all appearances identical with those of the peritoneum, from which they undoubtedly originate. This resemblance applies both to the character of their nuclei and to their lack of definite cell boundaries. The peritoneal layer and underlying mesenchyma are separated from one another by the basement membrane of the former. This is formed by the interlacing of protoplasmic fibrils given off by the cells of both layers. In later stages, one finds a progressive crowding of the peritoneal nuclei. In the rete and sex gland regions this results in the formation of tubular peritoneal invaginations, which are limited from the surrounding mesenchyma by the persistent membrana propria. In the sex gland region these tubular cords of cells may be known as the sex-cords. At this indifferent stage they are closely massed together side by side, and the very narrow interspaces between them contain scattered mesenchyme cells, which from now on, may be considered under the general term of stroma. This is used to designate the loose connective tissue of both ovary and testis. Invaginations, in all respects similar to these sex-cords, arise from the peritoneum of the rete region. They lie further

apart than do the sex-cords, and penetrate more deeply into the mesenchyme. Both rete-cords and sex-cords are at this stage devoid of a lumen. In cross-section they show a limiting membrana propria, within which is a single layer of cells arranged with their bases attached to the membrana propria and their apices meeting at a common point in the center — the rudiment of the lumen.

The mesentery of the sex gland is formed by the proliferation of connective tissue cells from the peritoneum, in areas of restricted width immediately ventral and dorsal to the sex gland. The ventral area is by far the more important of the two sources. The albuginea is in large part formed from the cells comprising the proximal parts of the sex-cords. They are formed at the time when the sex gland has begun to assume definite shape. At this time the rapidly dividing cells of the attached ends of the sex-cords have become differentiated from those of the more distal portions in that they elongate, become smaller, and acquire the property of staining more deeply. They then break away from the peritoneum on the one hand, and from the sex-cords on the other. They may still for some time be found attached to portions of the membrana propria that ensheathed them. They become mingled with certain exactly similar intertubular mesenchymal elements, to form the albuginea, which is essentially one with the remaining connective tissue or stroma of the sex gland. Sexual differentiation is first manifested by the cessation of growth of the sex-cords of the ovary. We can then distinguish them as medullary-cords. The peritoneum of the ovary begins to increase in thickness, and eventually forms the cortex of the adult ovary in a manner to be briefly indicated below. The albuginea of the ovary forms a broader, looser, and more irregular layer than does that of the testis. In the testis the peritoneum ceases to grow, in large measure at least, its cells becoming flattened, and in later life practically disappearing. The rete cords grow backwards from their points of origin, and enter the anterior part of the sex gland. They branch and anastomose throughout their course, sending branches to the Malpighian corpuscles on the one hand, and on the other to the seminiferous tubules of the testis. The branches passing to the Malpighian corpuscles

meet evaginations from the capsules of Bowman, with which they fuse. Such evaginations are irregular in number, as many as three having been counted upon the same glomerulus. Some glomeruli may send out none at all. The tubuli recti connecting the rete-cords with the seminiferous tubules are likewise irregular in number, being apparently called forth wherever needed.

Seminiferous tubules, medullary-cords, and rete-cords are homologous structures. Not only are they of similar origin, but their component cells show similarities. Two kinds of cells are found in all three structures: (1) primitive sex cells, which have already been described; (2) cells more or less variable but agreeing with one another in not having clearly marked cell limits, and also in the absence of centrosphere and centrosome. The cells of this second class form the germinative cells of the seminiferous tubules, the follicular cells of the medullary-cords, and the rete cells proper of the rete-tubules.

Returning to the subject of the ovary, the peritoneum at the time of separation of the medullary-cords, contains no differentiated sex cells. Such may exist, but they are at all events indistinguishable from the remaining peritoneal cells. The peritoneum or germinal epithelium, as it may now be termed, next begins to give rise to the cords of Pflüger, which branch and anastomose in a similar manner to the medullary-cords. Some of these cords of Pflüger may contain a well-defined lumen, in the case of advanced embryos of the pig (15 cm. length). In these later stages the inner ends of the cords are broken up to form follicles. Follicles are likewise formed in medullary-cords. These however, have never more than one layer of follicular cells in the forms studied.

The rete-cords come in contact with the medullary-cords, and are then scarcely distinguishable from the latter in the case of the rabbit. They contain no sex cells in later stages of the ovary of that animal, although such are present in the rete tissue when it is first laid down. By this criterion alone can one, in a very general way, distinguish between medullary-cords and rete-tissue lying within the rabbit's ovary. In the pig, on the other hand, the rete-tissue shows some very interesting characteristics. The portions of the rete-cords lying within the

ovary undergo similar differentiation to the medullary-cords and the cords of Pflüger forming the cortex. The rete-tissue within the ovary of the 18 cm. pig embryo is found to contain young follicles, each with a single layer of follicular cells; the enlarged oöcyte in the center having passed through the synapsis condition, characteristic of one stage in the development of the young oöcytes. All such follicles subsequently degenerate. In the testis the intra-glandular portions of the rete-tubules are similar to the seminiferous-tubules, but differ from them in their much smaller diameter and in the earlier acquisition of a lumen. They contain the sex cells characteristic of the seminiferous-tubules. These are at first present in the extra-glandular region of both ovary and testis, but disappear more or less completely in later stages. No attempt was made to study out the fate of the sex cells of the rete-tubules of the pig testis. They are still present in the 25 cm. pig embryo. In the rabbit they are found in the rete of the testis twenty-four days after birth, but are not to be found in that of a rabbit killed 140 days after birth. The rete-tubules are so completely united by anastomosis that their connected lumen forms a large irregular cavity divided here and there by irregular partitions formed by the walls of the several rete-tubules.

The connective tissue elements of ovary and testis are derived from the peritoneum. In early stages they are not distinguishable from the cells that make up the sex-cords, except that the latter are marked off from the stroma by their membrana propria. As before stated, the albuginea is largely formed by actual transformation of the basal part of the sex cords into connective tissue elements.

The interstitial cells are characterized by a large nucleus, distinct cell boundaries, a centrosphere and centrosome, and very granular cytoplasm. They first appear in the stroma of both testis and ovary of the pig of 2.5 cm. length. They are far more numerous in the testis than in the ovary. Their appearance is coincident with that of a large number of fatty globules in the peritoneum and sex cords. In the testis they persist for a long time. In the ovary, however, the few cells appearing at this stage speedily disappear. In both organs they divide by mitosis.

This process soon ceases in the ovary, while in the *testis*, on the other hand, division figures are found in the interstitial cells at a stage as late as the 7.5 cm. embryo. In the testis of the 15 cm. embryo, they have begun to degenerate. This process manifests itself in a shrinkage of the cytoplasm. Interstitial cells first form in the testis of the rabbit embryo of a stage between seventeen and twenty-one days. They are found to be still dividing by mitosis eight days after birth. They are very rare, however, in the stage of twenty-four days after birth.

No interstitial cells were found in the *ovary* of the embryo rabbit, they being first met with in females killed forty-five days after birth. Here they are scarce, but unmistakable. Considerable light is thrown upon their origin by a study of the eighty-five-day rabbit. In the ovary of this stage they are very common, their origin from the cells of the theca interna of atretic follicles being clearly shown. This, taken in connection with the additional fact that they make their appearance in the 2.5 cm. pig embryo coincident with the fatty degeneration of the germinative cells of the seminiferous tubules and their ovarian homologues, together with that of many cells of the germinal epithelium, would lead us to conclude that cell degeneration offers the stimulus or condition that brings about the formation of the interstitial cells.

Interstitial cells do not develop from unmodified connective tissue cells, such as those comprising the theca externa and the general ovarian stroma. Such stroma cells must be transformed into cells of the theca-interna by the direct or indirect influence of the growing follicles, before they are again susceptible to the influences exerted by the process of cell degeneration. Atresia of small follicles that are not surrounded by a theca-interna does not bring about the formation of interstitial cells. Many such small follicles are found to degenerate early and late in the history of the ovary.

No evidence has been found favoring the theory of the early segregation of the sex cells, but I am not prepared to say that my work has in any way tended to disprove such a theory. Sex cells appear in the very youngest stages studied (pig embryo, 0.6 cm. length and rabbit embryo of 13 days' age). They

are most common in the region where the sex-gland will eventually form, occurring both in the peritoneum and among scattered subperitoneal cells of mesenchymal nature. They are prominent in the sex-cords of a later stage. In the 1.8 cm. pig embryo, immediately after the separation of the sex-cords from the peritoneum, the latter is found to contain no sex-cells distinguishable as such. If the sex-gland be an ovary, they soon (2.5 cm. pig embryo) make their appearance in the peritoneum, and especially in the cords of Pflüger growing inward from it. These cords of Pflüger increase by growth at their bases, *i. e.*, their points of connection with the peritoneum. Hence there is a continual development of peritoneal cells to form the primitive ova distinguishable as such. The case of the seminiferous tubules is not so clear. Well-developed sex cells are found in them from the start. On the other hand, all stages of transition are found to link the germinative cells with the sex cells. These transitional cells are found in the testis of the pig at as late a stage as the 13 cm. embryo.

Whether the sex-cells that appear in the very early stages of embryonic development ever produce functional sex products in the testis, is a question that cannot easily be solved in this form. Certain it is, however, that true sex products do form in both ovary and testis from apparently undifferentiated cells of peritoneal origin, and that those which are functional in the ovary form *exclusively* from this source. The sex-glands and rete tissue are the seat of extensive processes of cell degeneration. I shall not here enter upon a discussion of the different forms which this process assumes, but shall defer treatment of these considerations to the more complete account.

This piece of research has brought up many interesting facts, bearing upon questions touching upon the action of trophic stimuli in embryonic development. Perhaps the most striking example of this is the formation of follicles in that portion of the rete tissue lying within the ovary. The extra-ovarian part, or that remaining in the mesonephros, does not contain follicles, although it is of precisely the same origin as the intra-ovarian portion. The influence of the ovary reaches out a short distance into the mesonephros, as can be seen by the presence there of a

few sex cells, which are more numerous in the regions nearest to the ovary. There is a definite interaction between the capsules of Bowman and the rete-cords lying nearest to them. Each sends forth a process to meet the other. In the testis the rete cords send out processes (tubuli recti) to meet the seminiferous tubules at their inner ends. Each tubule receives its rete branch. A large number of tubuli recti can arise, from a single rete-tubule at different points in its course. The connection between cell degeneration and the formation of interstitial cells has already been discussed. The uriniferous tubules of those glomeruli with which the rete-cords come in contact persist as the rete efferentia of the male, while the remaining ones disappear wholly, or in large part at least. A few rudiments of these rete efferentia tubules persist in contact with the rete ovarii. Such rudiments are very rare in the pig embryo of 25 cm. length.

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